NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for 4-NITROPYRENE

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Prepared by

Integrated Laboratory Systems
Post Office Box 13501
Research Triangle Park, North Carolina 27709
NIEHS Contract No. N01-ES-25346

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NTP Report on Carcinogens Listing for 4-Nitropyrene

Carcinogenicity

4-Nitropyrene is *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation at multiple tissue sites in multiple species of experimental animals (reviewed in IARC, 1989).

Intraperitoneal (i.p.) injections of 4-nitropyrene caused an increased incidence of liver tumors in male mice, lung tumors in male and female mice (Wislocki et al., 1986; cited by IARC, 1989), and mammary adenocarcinomas in female rats (Imaida et al., 1991). When administered by subcutaneous (s.c.) injections, 4-nitropyrene induced sarcomas at the injection site, and increased incidences in mammary adenocarcinomas, leukemia and tumors of the Zymbal gland in female rats (Imaida et al., 1995; IARC, 1989). In two studies, female rats receiving mammary gland injections of 4-nitropyrene showed an increased incidence of mammary tumors (Imaida et al., 1991; El-Bayoumy et al., 1993).

There are no adequate data available to evaluate the carcinogenicity of 4-nitropyrene in humans.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Although not as reactive/potent as some of the other mononitropyrenes or dinitropyrenes, 4-nitropyrene is genotoxic in bacterial cells and induces cell transformation in BALB-c cells *in vitro*. Metabolic pathways for 4-nitropyrene, leading to mutagenic and likely DNA adducts, have also been described (IARC, 1989).

No data are available that would suggest that the mechanisms thought to account for tumor induction by 4-nitropyrene in experimental animals would not also operate in humans.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded; or

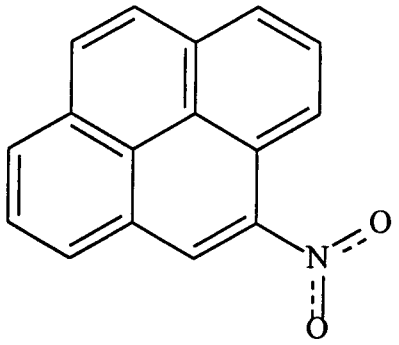
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgement, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 INTRODUCTION

4-Nitropyrene
[57835-92-4]



1.1 Chemical Identification

4-Nitropyrene ($C_{16}H_9NO_2$, mol. wt. = 247.25) is also called:

Pyrene, 4-nitro-
4-NP

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	Orange	Bavin (1959; cited by IARC, 1989)
Physical State	Slender needles	Bavin (1959; cited by IARC, 1989)
Melting Point, °C	196-197.5	Bavin (1959; cited by IARC, 1989)

1.3 Identification of Structural Analogues and Metabolites

Structural analogues and metabolites discussed in this report include the following:

4-Aminopyrene (4-AP, $C_{16}H_{11}N$, MW = 217.27)
trans-9,10-Dihydro-9,10-dihydroxy-4-nitropyrene
9(10)-Hydroxy-4-(acetylamino)pyrene [9(10)-OH-4-AAP]
9(10)-Hydroxy-4-nitropyrene
4-Nitropyrene-9,10-dione (4-NP-9,10-dione)
4-Nitropyrene-9,10-oxide (4-NP-9,10-oxide)

Physical-chemical properties could not be found for these compounds.

1.4 Report Organization

The rest of this report is organized into six additional sections (2.0 Human Exposure, 3.0 Human Studies, 4.0 Mammalian Carcinogenicity, 5.0 Genotoxicity, 6.0 Other Relevant Studies, and 7.0 References) and two appendixes. Appendix A describes the literature search in online databases, and Appendix B provides explanatory information for Figure 5-1. Tables and figures cited in the narrative follow the entire narrative for a section.

2.0 HUMAN EXPOSURE

2.1 Use

There is no evidence that 4-nitropyrene (4-NP) has been used for commercial applications (IARC, 1989).

2.2 Production

No evidence has been found that 4-NP has been produced for other than laboratory use (IARC, 1989). One U.S. company produces 4-NP (SRI, 1992). No data on imports or exports of 4-NP were available. No U.S. suppliers of 4-NP were identified by Chem Sources (Chem Sources, 1996).

2.3 Exposure

The primary route of potential human exposure to 4-NP is inhalation. Low concentrations of 4-NP were found in ambient airborne particulates in one study. Prior to 1980, some carbon black samples known to be used in photocopy machines were found to contain considerable quantities of nitropyrenes (IARC, 1989). 4-NP was not listed in the National Occupational Exposure Survey (1984) or the National Occupational Hazard Survey (1976) conducted by NIOSH.

2.4 Regulations

OSHA regulates 4-NP under the Hazard Communication Standard and as a chemical hazard in laboratories.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
OSHA	29 CFR 1910.1200. Promulgated 2/15/89. OSH Act: Hazard Communication Standard.	Requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication program to include labels, material safety data sheets, and worker training.
	29 CFR 1910.1450. Promulgated 1/31/90. Amended 55 FR 12111, 3/30/90. OSH Act: Final rule for occupational exposure to hazardous chemicals in laboratories.	As a select carcinogen (IARC Group 2B), 4-NP is included as a chemical hazard in laboratories. Employers required to provide employee information and training and to provide Chemical Hygiene Plan.

3.0 HUMAN STUDIES

No studies were found that evaluated the carcinogenicity of 4-NP in humans.

4.0 MAMMALIAN CARCINOGENICITY

Full details of mammalian carcinogenicity studies of 4-NP are presented in Table 4-1.

Summary: In newborn male and female mice treated with 4-NP, a significantly increased incidence and multiplicity of liver tumors (males only) and a significantly increased incidence of lung tumors (adenoma and carcinoma; males and females) was found. The incidences of mammary adenocarcinoma, Zymbal gland carcinoma, and leukemia were significantly increased in newborn female rats treated with 4-NP subcutaneously (s.c.), and the incidence of mammary adenocarcinoma, but not of fibroadenoma or adenoma, was significantly increased in 30-day-old female rats treated intraperitoneally (i.p.) with 4-NP. The incidences of mammary fibroadenoma and adenocarcinoma were significantly increased in 30-day-old female rats treated with 4-NP (administered as 2 doses; one dose injected in thoracic nipples and one dose injected in inguinal nipples).

4.1 Mice

Newborn male and female CD-1 mice treated with a 2.8-μmol total dose of 4-NP (administered as 3 intraperitoneal [i.p.] injections; 1 injection on days 1, 8, and 15 after birth) and evaluated at 1 year had a significantly increased incidence and multiplicity of liver tumors (males only) and a significantly increased incidence of lung tumors (adenoma and carcinoma; males and females) (Wislocki et al., 1986; cited by IARC, 1989).

4.2 Rats

4.2.1 Subcutaneous Injection

The incidences of mammary adenocarcinoma, Zymbal gland carcinoma, and leukemia were significantly increased in newborn female CD rats treated with 100 μmol 4-NP/kg mean body weight s.c. once per week for 8 weeks and evaluated for up to 86 weeks following treatment (King, 1988; cited by IARC, 1989; Imaida et al., 1995).

4.2.2 Intraperitoneal Injection

The incidence of mammary adenocarcinoma, but not of fibroadenoma or adenoma, was significantly increased in 30-day-old female CD rats treated with 67 μmol 4-NP/kg mean body weight intraperitoneally 3 times per week for 4 weeks and evaluated for up to 61 weeks after treatment (Imaida et al., 1991a).

4.2.3 Mammary Injection

The incidences of mammary fibroadenoma and adenocarcinoma were significantly increased in 30-day-old female CD rats treated with a total dose of 12.3 (Imaida et al., 1991a) or 4.06 (El-Bayoumy et al., 1993) μmol 4-NP (administered as 2 doses; one dose in thoracic nipples and one dose in inguinal nipples) when evaluate for up to 43 weeks or 77 weeks after treatment, respectively.

Table 4-1. Mammalian Carcinogenicity Studies of 4-Nitropyrene

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference(s)
Mice - *Intraperitoneal Injection*							
newborn CD-1 mouse	90 or 100 M and F [IARC, 1989 did not specify]	90 or 100 M and F [IARC, 1989 did not specify (DMSO alone) 90 or 100 M and Female [IARC, 1989 did not specify] (positive controls) A second vehicle control group was begun 10 wk after the first. Positive controls received 3 injections of B[*a*]P.	4-NP, > 99% pure	2.8 µmol total dose in 10, 20, and 40 µL DMSO, administered as 3 i.p. injections: on day 1, 8, and 15 after birth	15 days	Surviving mice were killed after 1 year. The statistical test used to analyze tumor incidence was not specified by IARC. **Liver:** Positive (for tumorigenesis; males only) The incidence of liver tumors was significantly increased in 4-NP-treated males (24/29 [4 adenomas, 20 carcinomas] vs. 2/28 males in control group 1 and 5/45 males in control group 2 [all adenomas]), but not 4-NP-treated females. It was also reported that the multiplicity of liver tumors was higher in 4-NP-treated males (6.0 nodules/tumor-bearing animal) as compared to controls (tumor multiplicity not specified). **Lung:** Positive (for tumorigenesis; males and females) The incidence of lung tumors in 4-NP-treated males (11/29 [10 adenomas, 1 carcinoma] vs. 1/28 males in control group 1 and 4/45 males in control group 2) and females (9/29 vs. 0/31 females in control group 1 and 2/34 females in control group 2) was significantly increased.	Wislocki et al. (1986; cited by IARC, 1989)

Table 4-1. Mammalian Carcinogenicity Studies of 4-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference(s)
Rats - Subcutaneous Injection							
newborn CD rat	[initial numbers unspecified]	[initial numbers unspecified]	4-NP, purity not specified	100 µmol/kg bw, once/wk, injected s.c.	8 wk	Rats were killed when moribund or after 86 weeks. All organs were examined macroscopically. The brain, pituitary gland, mammary glands, thyroid gland, esophagus, bronchus, lungs, stomach, small intestine, large intestine, liver, kidneys, spleen, ovaries, preputial gland, and any pathological lesions observed macroscopically were examined histologically. Statistical analysis of tumor incidence was performed using the χ^2 test. **Mammary Gland:** Positive (for tumorigenesis) The incidence of adenocarcinoma, but not of fibroadenoma or adenoma, was significantly increased in 4-NP-treated rats (18/27 vs. 0/47 controls; $p < 0.001$). The mean latency time for mammary tumors was 262 days for 4-NP-treated rats and 498 days for controls (significance not specified). **Blood:** Positive (for leukemia) The incidence of leukemia was significantly increased in 4-NP-treated rats (5/27 vs. 0/47 controls; $p < 0.005$). **Zymbal Gland:** Positive (for tumorigenesis) The incidence of Zymbal gland carcinoma was significantly increased in 4-NP-treated rats (4/27 vs. 0/47 controls; $p < 0.05$). **Injection Site.:** Positive (for tumorigenesis) The incidence of malignant fibrous histiocytoma was significantly increased in 4-NP-treated rats (10/27 vs. 0/47 controls; $p < 0.001$).	King (1988; cited by IARC, 1989) Imaida et al. (1995)

Table 4-1. Mammalian Carcinogenicity Studies of 4-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference(s)
Rats - *Intraperitoneal Injection*							
30-day-old CD rat	[initial numbers unspecified]	[initial numbers unspecified]	4-NP, purity not specified	67 µmol/kg bw in DMSO (25 µmol/mL DMSO), 3 times/wk, injected i.p.	4 wk	Rats were killed 61 weeks after the first treatment. All organs were examined macroscopically and histologically; "particular attention was given to the mammary glands." Statistical analysis was performed using χ^2 and Student's t-tests "wherever appropriate." **Mammary Gland:** Positive (for tumorigenesis) The incidence of adenocarcinoma, but not of fibroadenoma or adenoma, was significantly increased in 4-NP-treated rats (13/29 vs. 1/29 controls; $p < 0.005$). The multiplicity of mammary tumors (fibroadenoma, adenoma, adenocarcinoma) was not significantly increased in 4-NP-treated rats. The mean latency time of mammary tumors did not differ significantly between 4-NP-treated and control rats (272 vs. 318 days, respectively.	Imaida et al. (1991a)

Table 4-1. Mammalian Carcinogenicity Studies of 4-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference(s)
Rats - *Mammary Injection*							
30-day-old CD rat	30F	30F (DMSO alone)	4-NP, >99.8% pure	12.3 µmol total dose *day 1*: 100 µL of 4-NP solution (concentration not given) in DMSO injected directly into mammary tissue below each of 3 left thoracic nipples. Mammary tissue below right thoracic nipples injected with DMSO alone. *day 2*: 100 µL of 4-NP solution (concentration not given) injected directly into mammary tissue below inguinal nipples (side not specified).	2 days	Rats were killed 43 weeks after the second injection. All organs were examined histologically, with particular attention paid to mammary glands. All rats survived the duration of the experiment. Statistical analyses of tumor incidence and multiplicity were performed using the χ^2 and Student's *t*-test, respectively. **Mammary Gland:** Positive (for tumorigenesis) The incidences of fibroadenoma (13/30 vs. 5/30 controls; $p < 0.05$) and adenocarcinoma (7/30 vs. 0/30 controls; $p < 0.001$) were significantly increased in 4-NP-treated rats. The multiplicity of left- and right-side fibroadenomas and of left-side adenocarcinomas were significantly increased ($p < 0.001$) in 4-NP-treated rats. The number of adenocarcinomas on the left side was significantly greater than the number on the right side. 4-NP-treated rats had a significantly increased incidence of mammary tumors (all types combined) on both the left and right sides, compared to vehicle controls. Despite similarities in their experiments, the research groups of El-Bayoumy et al. (1993) and Imaida et al. (1991a) [see next page] have no members in common.	El-Bayoumy et al. (1993)

Table 4-1. Mammalian Carcinogenicity Studies of 4-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference(s)
30-day-old CD rat	[initial numbers unspecified]	[initial numbers unspecified]	4-NP, purity not specified	*day 1*: 100 µL of 20.3 µmol/mL DMSO (2.03 µmol 4-NP) injected directly into each of 3 left thoracic nipples. Right thoracic nipples injected with DMSO alone. *day 2*: 100 µL of 20.3 µmol/mL DMSO (2.0 µmol 4-NP) injected directly into inguinal nipples (side not specified).	2 days	Surviving rats were killed after 77 weeks. All organs were examined macroscopically and histologically; "particular attention was given to the mammary glands". Statistical analysis of tumor incidence was performed using the χ^2 test. **Mammary Gland:** Positive (for tumorigenesis) The incidences of fibroadenoma (15/28 vs. 5/28 controls; $p < 0.01$) and adenocarcinoma (19/28 vs. 1/28 controls; $p < 0.001$) were significantly increased in 4-NP-treated rats. The incidence of these tumors was significantly increased on the left side ($p < 0.001$), but not on the right side. The multiplicity of tumors did not differ significantly between 4-NP-treated and control rats.	Imaida et al. (1991a)

5.0 GENOTOXICITY

Studies of the genotoxic effects of 4-NP are summarized in Table 5-1.

Summary: Though not extensively tested, 4-NP was found to be positive in a number of prokaryotic and mammalian *in vitro* test systems [see Genetic Activity Profile, Figure 5-1 (data limited to IARC, 1989)]. When tested *in vitro*, 4-NP was found to induce DNA damage in *Bacillus subtilis,* gene mutations in *Salmonella typhimurium,* and cell transformation in mouse BALB/3T3 cells. It was negative for cell transformation in rat tracheal epithelial cells. Unless otherwise specified, rat liver S9 was the source of metabolic activation *in vitro.*

Information for studies reviewed in IARC Vol. 46, 1989 was limited to qualitative data with information on study design, doses tested, chemical purity, etc., not provided. For simplicity, multiple citations in IARC for the same genetic toxicity endpoint and test system are presented as a group rather than cited individually.

5.1 Noneukaryotic Systems

5.1.1 DNA Damage

In a review by IARC (1989), Horikawa et al. (1986) and Tokiwa et al. (1987) both reported that 4-NP at 0.01 to 2.0 μg/disc (0.04 to 8.0 nmol/disc) inhibited the growth of DNA repair-deficient strains of *B. subtilis* in the absence of metabolic activation (LED = 0.01 μg/disc; 0.04 nmol/disc).

5.1.2 Gene Mutations

As reported by Fu et al. (1985; cited by IARC, 1989), 4-NP (doses not given) was found to give positive results for *his* gene mutations in *S. typhimurium* strains TA98 and TA100 in the absence of S9 activation. In a later study by Busby et al. (1994), 4-NP at 0.1 to 5.0 μg/mL (0.4 to 20 μM) for 2 hours induced 8-azaguanine-resistant mutants in *S. typhimurium* strain TM677 both with and without metabolic activation, although it was substantially less mutagenic in the presence of S9 than in its absence [LED = 2.5 μg/mL (10 μM) +S9; 0.3 μg/mL (1.2 μM) -S9].

5.2 Mammalian Systems *In Vitro*

West and Rowland (1994) reported that 4-NP at 5.25 to 11.5 μg/mL (21.2 to 46.5 μM) for 24 hours did not induce morphological transformations in male Fischer 344 rat tracheal epithelial cells. Sheu et al. (1994), however, later reported that 4-NP at 0.8 to 20.0 μg/mL (3.0 to 80.9 μM) for a 48-hour exposure period induced a reproducible increase in morphological transformations in mouse BALB/3T3 cells clone A31-1-1 [LED = 20.0 μg/mL (80.9 μM)].

Table 5-1. Summary of 4-Nitropyrene Genotoxicity Studies

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference(s)
5.1 Noneukaryotic Systems							
5.1.1 DNA Damage							
Bacillus subtilis (strains not provided)	growth inhibition of DNA repair-deficient strains	-	n.p.	n.g.	positive	LED = 0.01 μg/disc (0.04 nmol/disc)	Horikawa et al. (1986) & Tokiwa et al. (1987; cited by IARC, 1989)
5.1.2 Gene Mutations							
Salmonella typhimurium strains TA98 and TA100	*his* gene mutations	-	n.p.	n.g.	positive	Dose levels and study design were not given.	Fu et al. (1985; cited by IARC, 1989)
S. typhimurium strain TM677	gene mutations (resistance to 8-azaguanine)	+/-	>99%	0.1 to 5.0 μg/mL (0.4 to 20 μM) for 2 h	positive/ positive	4-NP was substantially less mutagenic in the presence of S9 than in its absence. LED = 2.5 μg/mL (10 μM) +S9, 0.3 μg/mL (1.2 μM) -S9.	Busby et al. (1994)
5.2 Mammalian Systems *In Vitro*							
5.2.1 Cell Transformation							
male Fischer 344 rat tracheal epithelial cells	cell transformation	NA	>99%	5.25 to 11.5 μg/mL (21.2 to 46.5 μM) for 24 h	negative	Little relationship was seen between increasing dose and transformation frequency.	West and Rowland (1994)
mouse BALB/3T3 cells clone A31-1-1	cell transformation	NA	>99%	0.8 to 20.0 μg/mL (3 to 80.9 μM) for 48 h	positive	4-NP induced a reproducible positive response, LED = 20.0 μg/mL (80.9 μM).	Sheu et al. (1994)

Abbreviations: HID = highest ineffective dose; LED = lowest effective dose; n.p .= purity not provided; n.g. = doses not given; neg. = negative; pos. = positive

Figure 5-1. Genetic Activity Profile of 4-Nitropyrene (Data limited to IARC, 1989)

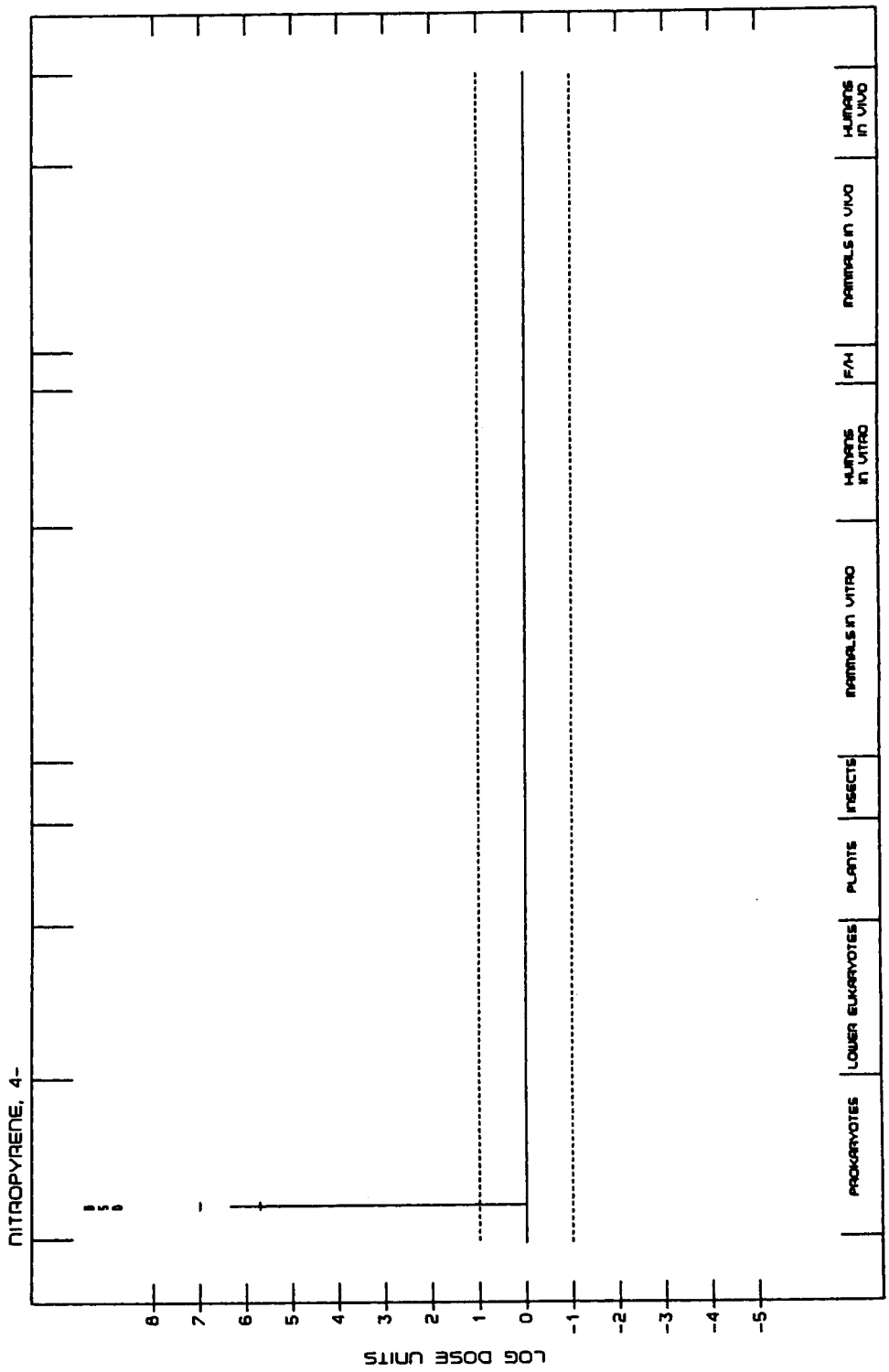
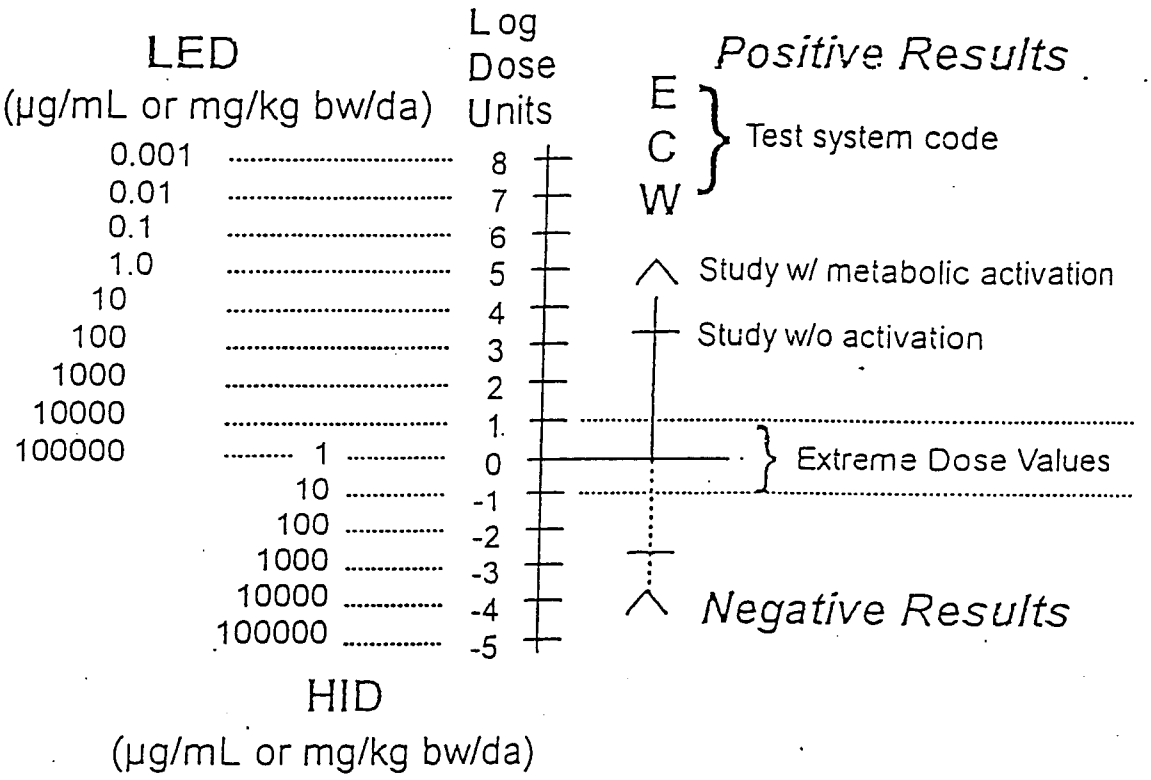


Figure 5-2. Schematic View of a Genetic Activity Profile (GAP)



A schematic view of a Genetic Activity Profile (GAP) representing four studies (two positive and two negative) for an example short-term test, ECW. Either the lowest effective dose (LED) or the highest ineffective dose (HID) is recorded from each study, and a simple mathematical transformation (as illustrated above) is used to convert LED or HID values into the logarithmic dose unit (LDU) values plotted in a GAP. For each test, the average of the LDUs of the majority call is plotted using a solid vertical bar drawn from the origin. A dashed vertical bar indicates studies that conflict with the majority call for the test. Note in cases where there are an equal number of positive and negative studies, as shown here, the overall call is determined positive. The GAP methodology and database have been reported previously (Garrett et al., 1984; Waters et al., 1988, 1991).

Garrett, N.E., H.F. Stack, M.R. Gross, and M.D. Waters. 1984. An analysis of the spectra of genetic activity produced by known or suspected human carcinogens. Mutat. Res. 143:89-111.

Waters, M.D., H.F. Stack, A.L. Brady, P.H.M. Lohman, L. Haroun, and H. Vainio. 1988. Use of computerized data listings and activity profiles of genetic and related effects in the review of 195 compounds. Mutat. Res. 205:295-312.

Waters, M.D., H.F. Stack, N.E. Garrett, and M.A. Jackson. 1991. The genetic activity profile database. Environ. Health Perspect. 96:41-45.

6.0 OTHER RELEVANT STUDIES

6.1 Absorption, Distribution, Metabolism, Excretion

Summary: When incubated under aerobic conditions with male rat liver microsomes that had been pretreated with 3-methylcholanthrene (3-MC), 4-NP was metabolized to 4-nitropyrene-9,10-dione (4-NP-9,10-dione; major metabolite). Another metabolite was present, but its structure could not be determined. The only metabolite detected when 4-NP was incubated with an epoxide hydrolase inhibitor and rat liver microsomes was the epoxy derivative 4-nitropyrene-9,10-oxide (4-NP-9,10-oxide). In contrast to *in vitro* metabolism, *in vivo* metabolism of 4-NP may proceed via both nitroreduction and ring oxidation.

4-Aminopyrene and 9(10)-hydroxy-4-(acetylamino)pyrene were identified in fecal material excreted from female rats administered 4-NP orally. Forty-eight hours following oral administration of 4-NP, 32% of the dose was recovered in the urine and 30.6% was recovered in the feces.

6.1.1 Absorption

No studies were found that described the absorption of 4-NP.

6.1.2 Distribution

No studies were found that described the distribution of 4-NP.

6.1.3 Metabolism

The first in-depth study of the metabolism of 4-NP was conducted by Upadhyaya et al. (1994). They performed both *in vitro* and *in vivo* assays. For the *in vitro* tests, 4-NP was incubated under aerobic conditions with male rat liver microsomes that had been pretreated with 3-MC. 4-NP and its metabolites were extracted with ethyl acetate (EtOAc) and separated using HPLC. A major metabolite was eluted and NMR analysis revealed it to be 4-nitropyrene-9,10-dione (4-NP-9,10-dione). Another metabolite was present, but its structure could not be determined. 4-NP was also incubated with male rat liver microsomes in the presence of the epoxide hydrolase inhibitor 3,3,3-trichloropropylene-1,2-oxide (TCPO). The only metabolite detected was the epoxy derivative 4-NP-9,10-oxide.

At the time (1994), it was not known how 4-NP-9,10-dione was formed, but the authors suggested that hydrolysis of 4-NP-9,10-oxide, followed by oxidation (via an enzymatic or chemical reaction) of the *trans*-9,10-dihydro-9,10-dihydroxy-4-nitropyrene intermediate was a possibility.

An *in vivo* study revealed other metabolites of 4-NP and showed that, in contrast to *in vitro* metabolism, *in vivo* metabolism of 4-NP may proceed via both nitroreduction and ring oxidation. Forty-eight hours following oral administration of [^{3}H]4-NP to female Sprague-Dawley rats, 4-aminopyrene and 9(10)-hydroxy-4-(acetylamino)pyrene [9(10)-OH-4-AAP] were identified in fecal material as 5.4% and 3.3% of the original dose, respectively. Unmetabolized 4-NP accounted for 2.4% of the original dose. Other metabolites were detected but could not be identified, although one was thought to be a phenolic compound. Organic-solvent-extractable metabolites in urine were identified as 4-AP (0.3%), 9(10)-OH-4-AAP (0.2%) and 9(10)-OH-4-NP (0.8%). Unmetabolized 4-NP accounted for 0.2% of the original dose. To isolate water-soluble urinary metabolites, urine was extracted with EtOAc and incubated for 6 hours in pH 5.5

buffer with β-glucuronidase or arylsulfatase in the presence of saccharic acid 1,4-lactone. Water-soluble urinary metabolites were identified as glucuronide (1.4% of dose) and sulfate (2.5% of dose) conjugates of 9(10)-OH-4-AAP.

6.1.4 Excretion

A study by Upadhyaya et al. (1994) revealed that urinary excretion of 4-NP by female Sprague-Dawley rats slightly exceeded gastrointestinal excretion of 4-NP after oral dosing. Forty-eight hours following oral administration of 58 mg 4-NP/kg bw, 32% of the dose was recovered in the urine and 30.6% was recovered in the feces. This elimination/excretion pattern was shown to be quite different from that of 1-NP and 2-NP, structural isomers of 4-NP. These two compounds were eliminated mainly in the feces of the exposed rats and only slightly in the urine.

6.2 Pharmacokinetics/Pharmacodynamics

No studies were found that described the pharmacokinetics or pharmacodynamics of 4-NP.

6.3 Modes of Action

No studies were found that described the mechanism(s) by which 4-NP is carcinogenic in rodents, and only very limited data are available on the genotoxicity of 4-NP. As reviewed in Section 5.0, 4-NP induced DNA damage in *B. subtilis*, was mutagenic in *Salmonella,* and induced cell transformation in mouse BALB/3T3 cells, but not in rat tracheal epithelial cells. It is reasonable to expect that the metabolic pathways responsible for the activation of 1-NP and 1,6- and 1,8-DNP to reactive species capable of inducing DNA damage and mutations in prokaryote and eukaryote cells would also activate 4-NP to genotoxic metabolites.

6.4 Structure-Activity Relationships

Summary: It has been reported that the mutagenicities of various nitroarenes were bilinearly related to the hydrophobicity of the compounds, with an optimal hydrophobicity constant (log P) of 5.44. An increase in the mutagenicity of nitropyrenes towards *S. typhimurium* as the number of nitro groups per compound increased was also found. Another study showed that a linear relationship existed between the first half-wave potential ($E_{1/2}$) and the logarithms of the mutagenicities of various nitroarenes towards *S. typhimurium*. Computer Automated Structure Evaluation (CASE) found two activating and 2 deactivating structures that were reported to be involved in the mediation of nitroarene mutagenicity in *S. typhimurium* (see Figure 6-1).

The mutagenic and carcinogenic potentials of nitroarene analogues vary. Some analogues are mutagenic and genotoxic in many systems, while other analogues are only mutagenic in some systems or are not mutagenic at all (for review see Klopman and Rosenkranz, 1984). Furthermore, some analogues are carcinogenic in rodents, while other analogues are not (Rosenkranz, 1987).

Several studies have been performed that identify structure-activity relationships among the nitroarenes. For example, Mermelstein et al. (1985) reported that there was an increase in the mutagenicity of nitropyrenes (exogenous metabolic activation not mentioned) towards *S. typhimurium* strains TA98 and TA98NR as the number of nitro groups per compound increased.

Mutagenicity towards these 2 strains increased in the order: 1-NP < 1,3-DNP < 1,6-DNP < 1,8-DNP. However, with further addition of nitro groups, the mutagenic activity of nitropyrenes decreased. After reaching a maximum with 1,8-DNP, mutagenicity declined for 1,3,6-trinitro- and 1,3,6,8-tetranitropyrene. In other strains of *S. typhimurium* (TA1537 and TA1538), the mutagenicity of 1-NP was 2 to 3 orders of magnitude lower than the mutagenicities of di-, tri-, and tetranitropyrenes, but within the di-, tri-, and tetranitropyrenes there was no apparent pattern for increasing mutagenicity.

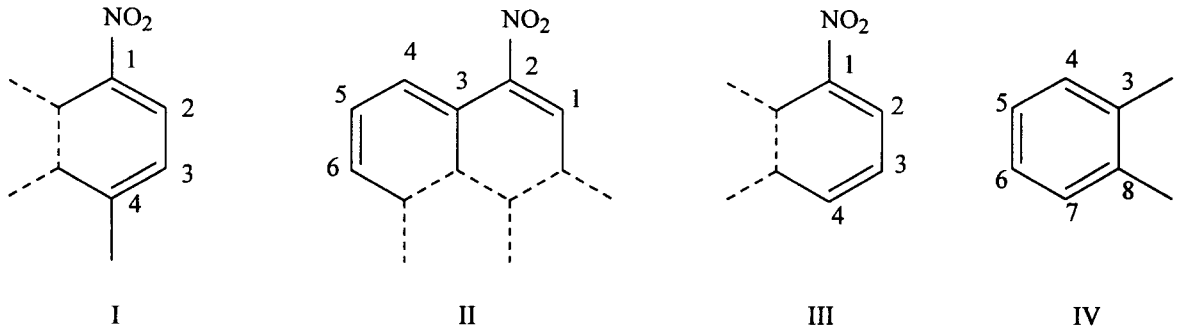
Klopman et al. (1984) reported that a linear relationship existed between the first half-wave potential ($E_{1/2}$) and the logarithms of the mutagenicities of various nitroarenes (including 1-NP, 1,6-DNP, and 1,8-DNP) towards *S. typhimurium* strains TA98 and TA1538. The lower the $E_{1/2}$, the more readily the nitroarene was nitroreduced. It was not stated whether exogenous metabolic activation was used. Since a linear relationship was also found to exist between $E_{1/2}$ and the calculated energies of the lowest unoccupied molecular orbital (LUMO), the authors suggested that the mutagenicities of nitroarenes could be predicted from their calculated LUMO energies. Debnath et al. (1992) reported that the mutagenic activity of aromatic and heteroaromatic nitro compounds (including 1-NP, 4-NP, 1,6-DNP, 1,8-DNP, and 6-NC) towards *S. typhimurium* strain TA100, without exogenous metabolic activation, was also linearly related to the LUMO energies of the compounds. Debnath et al. (1992) also reported that the mutagenicities of various nitroarenes were bilinearly related to the hydrophobicity of the compounds, with an optimal hydrophobicity constant (log P) of 5.44.

Klopman and Rosenkranz (1984) used the Computer Automated Structure Evaluation (CASE) program to predict the mutagenicity (without exogenous metabolic activation) of 53 nitroarenes (including 1-NP, 1,6-DNP, 1,8-DNP, and 6-NC) towards *S. typhimurium* strain TA98. Two activating and 2 deactivating structures were reported to be involved in the mediation of nitroarene mutagenicity (see Figure 6-1).

6.5 Cell Proliferation

No studies were found that evaluated the effect of 4-NP on cell proliferation.

Figure 6-1. Fragments Responsible for the Mutagenicity of Nitroarenes



I II III IV

Fragments I and II are required for activity while fragments III and IV are deactivating. I differs from III in that C-4 is not bonded to a hydrogen (Klopman and Rosenkranz, 1984).

7.0 REFERENCES

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APPENDIX A

DESCRIPTION OF ONLINE SEARCHES FOR THE NITROARENES

DESCRIPTION OF ONLINE SEARCHES FOR THE NITROARENES (IARC Monograph in Vol. 46, 1989)

Online searching was done by the technical support contractor in TOXLINE January 30, 1996, using the CASRNs of the title compounds and *o*-nitroanisole and specifying publications after 1988. IARC (1989) was to be relied on for identification of pertinent earlier references. The 1240 records in TOXLINE were reduced by combining with the controlled vocabulary terms for metabolism and neoplasms and with the free-text truncated terms carcinogen? or mechanis? or toxicokinetic? or metab? From the 418 resulting records, the contractor selected approximately 160 for acquisition. Of the approximately 100 citations related to biological activity independently selected by the primary reviewer from NIEHS Review Group 1, 20 were identified as abstracts for which full publications were available; 73 had also been selected by the contractor. Thus, the primary reviewer selected 7 additional references that had not been identified as potentially useful by the contractor.

An exhaustive search of other pertinent toxicology databases was not attempted for the nitroarenes. A high degree of redundancy had been noted between TOXLINE and the databases CANCERLIT, EMBASE (Excerpta Medica), MEDLINE, and NIOSHTIC (Occupational Safety and Health). No special attempt was made to find toxicity information about metabolites and other structural analogues in the search strategy.

The contractor also searched CSCHEM and CSCORP for U.S. suppliers (Chem Sources databases); EMIC; EMICBACK; HSDB; IRIS; TSCATS (Toxic Substances Control Act Test Submissions); the Chemical Information System's databases SANSS (the Structure and Nomenclature Search System), ISHOW (for physical-chemical properties), and REGMAT (May 1993 version; this regulatory information database with broad coverage of EPA regulations is no longer available); Chemical Abstracts Service's (CAS) CA and Registry Files for metabolism studies (152 records) and metabolite identification; CAS File CHEMLIST for TSCA and SARA updates in 1996; and CA File sections 59 (Air Pollution and Industrial Hygiene), 60 (Waste Disposal and Treatment), and 61 (Water) for environmental exposure information. For current awareness, the contractor monitored Current Contents on Diskette® Life Sciences 1200 [journals] edition. Older literature that needed to be examined was identified from the reviews and original articles as they were acquired.

APPENDIX B

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

Test Code	Definition
ACC	Allium cepa, chromosomal aberrations
AIA	Aneuploidy, animal cells in vitro
AIH	Aneuploidy, human cells in vitro
ANF	Aspergillus nidulans, forward mutation
ANG	Aspergillus nidulans, genetic crossing-over
ANN	Aspergillus nidulans, aneuploidy
ANR	Aspergillus nidulans, reverse mutation
ASM	Arabidopsis species, mutation
AVA	Aneuploidy, animal cells in vivo
AVH	Aneuploidy, human cells in vivo
BFA	Body fluids from animals, microbial mutagenicity
BFH	Body fluids from humans, microbial mutagenicity
BHD	Binding (covalent) to DNA, human cells in vivo
BHP	Binding (covalent) to RNA or protein, human cells in vivo
BID	Binding (covalent) to DNA in vitro
BIP	Binding (covalent) to RNA or protein in vitro
BPF	Bacteriophage, forward mutation
BPR	Bacteriophage, reverse mutation
BRD	Other DNA repair-deficient bacteria, differential toxicity
BSD	Bacillus subtilis rec strains, differential toxicity
BSM	Bacillus subtilis multi-gene test
BVD	Binding (covalent) to DNA, animal cells in vivo
BVP	Binding (covalent) to RNA or protein, animal cells in vivo
CBA	Chromosomal aberrations, animal bone-marrow cells in vivo
CBH	Chromosomal aberrations, human bone-marrow cells in vivo
CCC	Chromosomal aberrations, spermatocytes treated in vivo and cytes obs.
CGC	Chromosomal aberrations, spermatogonia treated in vivo and cytes obs.
CGG	Chromosomal aberrations, spermatogonia treated in vivo and gonia obs.
CHF	Chromosomal aberrations, human fibroblasts in vitro
CHL	Chromosomal aberrations, human lymphocyte in vitro
CHT	Chromosomal aberrations, transformed human cells in vitro
CIA	Chromosomal aberrations, other animal cells in vitro
CIC	Chromosomal aberrations, Chinese hamster cells in vitro
CIH	Chromosomal aberrations, other human cells in vitro
CIM	Chromosomal aberrations, mouse cells in vitro
CIR	Chromosomal aberrations, rat cells in vitro
CIS	Chromosomal aberrations, Syrian hamster cells in vitro
CIT	Chromosomal aberrations, transformed animal cells in vitro
CLA	Chromosomal aberrations, animal leukocytes in vivo
CLH	Chromosomal aberrations, human lymphocytes in vivo

Test Code	Definition
COE	Chromosomal aberrations, oocytes or embryos treated in vivo
CVA	Chromosomal aberrations, other animal cells in vivo
CVH	Chromosomal aberrations, other human cells in vivo
DIA	DNA strand breaks, cross-links or rel. damage, animal cells in vitro
DIH	DNA strand breaks, cross-links or rel. damage, human cells in vitro
DLM	Dominant lethal test, mice
DLR	Dominant lethal test, rats
DMC	Drosophila melanogaster, chromosomal aberrations
DMG	Drosophila melanogaster, genetic crossing-over or recombination
DMH	Drosophila melanogaster, heritable translocation test
DML	Drosophila melanogaster, dominant lethal test
DMM	Drosophila melanogaster, somatic mutation (and recombination)
DMN	Drosophila melanogaster, aneuploidy
DMX	Drosophila melanogaster, sex-linked recessive lethal mutation
DVA	DNA strand breaks, cross-links or rel. damage, animal cells in vivo
DVH	DNA strand breaks, cross-links or rel. damage, human cells in vivo
ECB	Escherichia coli (or E. coli DNA), strand breaks, cross-links or repair
ECD	Escherichia coli pol A/W3110-P3478, diff. toxicity (spot test)
ECF	Escherichia coli (excluding strain K12), forward mutation
ECK	Escherichia coli K12, forward or reverse mutation
ECL	Escherichia coli pol A/W3110-P3478, diff. toxicity (liquid susp. test)
ECR	Escherichia coli, miscellaneous strains, reverse mutation
ECW	Escherichia coli WP2 uvrA, reverse mutation
EC2	Escherichia coli WP2, reverse mutation
ERD	Escherichia coli rec strains, differential toxicity
FSC	Fish, chromosomal aberrations
FSI	Fish, micronuclei
FSM	Fish, mutation
FSS	Fish, sister chromatid exchange
FSU	Fish, unscheduled DNA synthesis
GCL	Gene mutation, Chinese hamster lung cells exclusive of V79 in vitro
GCO	Gene mutation, Chinese hamster ovary cells in vitro
GHT	Gene mutation, transformed human cells in vivo
GIA	Gene mutation, other animal cells in vitro
GIH	Gene mutation, human cells in vitro
GML	Gene mutation, mouse lymphoma cells exclusive of L5178Y in vitro
GVA	Gene mutation, animal cells in vivo
G5T	Gene mutation, mouse lymphoma L5178Y cells in vitro, TK locus
G51	Gene mutation, mouse lymphoma L5178Y cells in vitro, all other loci
G9H	Gene mutation, Chinese hamster lung V-79 cells in vitro, HPRT locus
G9O	Gene mutation, Chinese hamster lung V-79 cells in vitro, ouabain resistance
HIM	Haemophilus influenzae, mutation
HMA	Host mediated assay, animal cells in animal hosts

Test Code	Definition
HMH	Host mediated assay, human cells in animal hosts
HMM	Host mediated assay, microbial cells in animal hosts
HSC	Hordeum species, chromosomal aberrations
HSM	Hordeum species, mutation
ICH	Inhibition of intercellular communication, human cells in vitro
ICR	Inhibition of intercellular communication, rodent cells in vitro
KPF	Klebsiella pneumonia, forward mutation
MAF	Micrococcus aureus, forward mutation
MHT	Mouse heritable translocation test
MIA	Micronucleus test, animal cells in vitro
MIH	Micronucleus test, human cells in vitro
MST	Mouse spot test
MVA	Micronucleus test, other animals in vivo
MVC	Micronucleus test, hamsters in vivo
MVH	Micronucleus test, human cells in vivo
MVM	Micronucleus test, mice in vivo
MVR	Micronucleus test, rats in vivo
NCF	Neurospora crassa, forward mutation
NCN	Neurospora crassa, aneuploidy
NCR	Neurospora crassa, reverse mutation
PLC	Plants (other), chromosomal aberrations
PLI	Plants (other), micronuclei
PLM	Plants (other), mutation
PLS	Plants (other), sister chromatid exchanges
PLU	Plants, unscheduled DNA synthesis
PRB	Prophage, induction, SOS repair, DNA strand breaks, or cross-links
PSC	Paramecium species, chromosomal aberrations
PSM	Paramecium species, mutation
RIA	DNA repair exclusive of UDS, animal cells in vitro
RIH	DNA repair exclusive of UDS, human cells in vitro
RVA	DNA repair exclusive of UDS, animal cells in vivo
SAD	Salmonella typhimurium, DNA repair-deficient strains, differential toxicity
SAF	Salmonella typhimurium, forward mutation
SAL	Salmonella typhimurium, all strains, reverse mutation
SAS	Salmonella typhimurium (other misc. strains), reverse mutation
SA0	Salmonella typhimurium TA100, reverse mutation
SA1	Salmonella typhimurium TA97, reverse mutation
SA2	Salmonella typhimurium TA102, reverse mutation
SA3	Salmonella typhimurium TA1530, reverse mutation
SA4	Salmonella typhimurium TA104, reverse mutation
SA5	Salmonella typhimurium TA1535, reverse mutation
SA7	Salmonella typhimurium TA1537, reverse mutation
SA8	Salmonella typhimurium TA1538, reverse mutation

Test Code	Definition
SA9	Salmonella typhimurium TA98, reverse mutation
SCF	Saccharomyces cerevisiae, forward mutation
SCG	Saccharomyces cerevisiae, gene conversion
SCH	Saccharomyces cerevisiae, homozygosis by recombination or gene conversion
SCN	Saccharomyces cerevisiae, aneuploidy
SCR	Saccharomyces cerevisiae, reverse mutation
SGR	Streptomyces griseoflavus, reverse mutation
SHF	Sister chromatid exchange, human fibroblasts in vitro
SHL	Sister chromatid exchange, human lymphocytes in vitro
SHT	Sister chromatid exchange, transformed human cells in vitro
SIA	Sister chromatid exchange, other animal cells in vitro
SIC	Sister chromatid exchange, Chinese hamster cells in vitro
SIH	Sister chromatid exchange, other human cells in vitro
SIM	Sister chromatid exchange, mouse cells in vitro
SIR	Sister chromatid exchange, rat cells in vitro
SIS	Sister chromatid exchange, Syrian hamster cells in vitro
SIT	Sister chromatid exchange, transformed cells in vitro
SLH	Sister chromatid exchange, human lymphocytes in vivo
SLO	Mouse specific locus test, other stages
SLP	Mouse specific locus test, postspermatogonia
SPF	Sperm morphology, F1 mouse
SPH	Sperm morphology, human
SPM	Sperm morphology, mouse
SPR	Sperm morphology, rat
SPS	Sperm morphology, sheep
SSB	Saccharomyces species, DNA breaks, cross-links or related damage
SSD	Saccharomyces cerevisiae, DNA repair-deficient strains, diff. toxicity
STF	Streptomyces coelicolor, forward mutation
STR	Streptomyces coelicolor, reverse mutation
SVA	Sister chromatid exchange, animal cells in vivo
SVH	Sister chromatid exchange, other human cells in vivo
SZD	Schizosaccharomyces pombe, DNA repair-deficient strains, diff. toxicity
SZF	Schizosaccharomyces pombe, forward mutation
SZG	Schizosaccharomyces pombe, gene conversion
SZR	Schizosaccharomyces pombe, reverse mutation
T7R	Cell transformation, SA7/rat cells
T7S	Cell transformation, SA7/Syrian hamster embryo cells
TBM	Cell transformation, BALB/C3T3 mouse cells
TCL	Cell transformation, other established cell lines
TCM	Cell transformation, C3H10T1/2 mouse cells
TCS	Cell transformation, Syrian hamster embryo cells, clonal assay
TEV	Cell transformation, other viral enhancement systems
TFS	Cell transformation, Syrian hamster embryo cells, focus assay

Test Code	Definition
TIH	Cell transformation, human cells in vitro
TPM	Cell transformation, mouse prostate cells
TRR	Cell transformation, RLV/Fischer rat embryo cells
TSC	Tradescantia species, chromosomal aberrations
TSI	Tradescantia species, micronuclei
TSM	Tradescantia species, mutation
TVI	Cell transformation, treated in vivo, scored in vitro
UBH	Unscheduled DNA synthesis, human bone-marrow cells in vivo
UHF	Unscheduled DNA synthesis, human fibroblasts in vitro
UHL	Unscheduled DNA synthesis, human lymphocytes in vitro
UHT	Unscheduled DNA synthesis, transformed human cells in vitro
UIA	Unscheduled DNA synthesis, other animal cells in vitro
UIH	Unscheduled DNA synthesis, other human cells in vitro
UPR	Unscheduled DNA synthesis, rat hepatocytes in vivo
URP	Unscheduled DNA synthesis, rat primary hepatocytes
UVA	Unscheduled DNA synthesis, other animal cells in vivo
UVC	Unscheduled DNA synthesis, hamster cells in vivo
UVH	Unscheduled DNA synthesis, other human cells in vivo
UVM	Unscheduled DNA synthesis, mouse cells in vivo
UVR	Unscheduled DNA synthesis, rat cells (other than hepatocytes) in vivo
VFC	Vicia faba, chromosomal aberrations
VFS	Vicia faba, sister chromatid exchange